

University of Groningen

Single-molecule enzymology with a ClyA nanopore

Galenkamp, Nicole

DOI:
[10.33612/diss.130258760](https://doi.org/10.33612/diss.130258760)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Galenkamp, N. (2020). *Single-molecule enzymology with a ClyA nanopore*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.130258760>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Single-molecule enzymology with a ClyA nanopore

Nicole Stéphanie Galenkamp

ISBN: 978-94-028-2119-2

© 2020 by Nicole S. Galenkamp (Rijksuniversiteit Groningen)

The research covered in this thesis was conducted at the Department of Chemical biology at the Groningen Biomolecular Science and Biotechnology, Rijksuniversiteit Groningen.

This project was financially supported by European Research Council (ERC)

All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means without written permission by the author and the publisher holding the copyright of the published articles.

Cover design by Nicole Galenkamp
Printed and bound by IPSKAMP printing
Groningen, Nederland, 2020



rijksuniversiteit
 groningen

Single-molecule enzymology with a ClyA nanopore

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 24 augustus 2020 om 18.00 uur

door

Nicole Stéphanie Galenkamp

geboren op 26 oktober 1990
te Hardenberg

Promotor

Prof. dr. G. Maglia

Copromotor

Dr. M. Soskine

Beoordelingscommissie

Prof. dr. G.J. Poelarends

Prof. dr. J.G. Roelfes

Prof. dr. W. Huck

Table of content

1. Introduction and outline of thesis	5
1.1 Introduction	6
1.2 History of enzymes research	6
1.3 Single-molecule studies	10
1.4 Nanopores	19
1.5 Overview of single-molecule nanopore enzymology	24
1.6 Dihydrofolate reductase	28
1.7 Glucose binding protein	30
1.8 Scope of this thesis	33
1.9 References	34
2. Direct electrical quantification of glucose and asparagine from bodily fluids using nanopores	51
2.1 Introduction	52
2.2 Results	52
2.2.1 <i>Characterisation of the glucose-binding protein</i>	52
2.2.2 <i>Quantification of glucose from human biological fluids</i>	54
2.2.3 <i>Detection of asparagine</i>	55
2.2.4 <i>Simultaneous detection of glucose and asparagine in sweat</i>	56
2.3 Discussion	57
2.4 Material and methods	58
2.5 Supplementary tables and figures	65
2.6 References	71
3. Directional conformer exchange in dihydrofolate reductase revealed by single-molecule nanopore recordings	73
3.1 Introduction	74
3.2 Results	75
3.2.1 <i>Binding of ligands to nanopore-trapped DHFR</i>	75
3.2.2 <i>DHFR Conformers</i>	79
3.2.3 <i>Binding of methotrexate to DHFR conformers</i>	80
3.3 Discussion	82
3.4 Additional results and discussion	83
3.5 Materials and methods	86
3.6 Supplementary Tables and figures	94
3.7 References	103

4. Substrate binding and turnover modulate the affinity landscape of dihydrofolate reductase to increase its catalytic efficiency	109
4.1 Introduction	110
4.2 Results	112
4.2.1 <i>Binding of substrate ligands to DHFR_{tag}</i>	112
4.2.2 <i>Ternary complex formation from the closed conformation</i>	113
4.2.3 <i>Ternary complex formation from the occluded conformation: product release</i>	114
4.2.4 <i>Catalyzed reaction</i>	116
4.3 Discussion and Conclusion	117
4.4 Material and methods	121
4.5 Supplementary information	127
4.6 References	136
5. Ligand binding induces the conformational change in the glucose binding protein	141
5.1 Introduction	142
5.2 Results	144
5.2.1 <i>Intrinsic closing of GBP</i>	144
5.2.2 <i>Glucose binding kinetic scheme</i>	144
5.2.3 <i>Binding affinity of GBP mutants</i>	146
5.2.4 <i>Intrinsic dynamics of GBP mutants</i>	148
5.2.5 <i>A glucose sensor</i>	149
5.3 Discussion	149
5.4 Conclusion	152
5.5 Material and Methods	154
5.6 Supplementary Tables and figures	159
5.7 References	173
6. Concluding discussion and perspectives	179
6.1 Biosensors	180
6.2 Mutated forms of dihydrofolate reductase	181
6.3 Dihydrofolate reductase from other species	181
6.4 Test more enzymes in pores	182
6.5 Computational research	183
6.6 New biological pores	183
6.7 References	184
7. Summary	187
8. Nederlandse samenvatting	191
9. Acknowledgements	197

10. List of publications	201
11. Curriculum vitae	203

